

Characterization of a Methane-Utilizing Bacterium from a Bacterial Consortium That Rapidly Degrades Trichloroethylene and Chloroform

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A mixed culture of bacteria grown in a bioreactor with methane as a carbon and energy source rapidly oxidized trichloroethylene and chloroform. The most abundant organism was a crescent-shaped bacterium that bound the fluorescent oligonucleotide signature probes that specifically hybridize to serine pathway methylo-trophs. The 5S rRNA from this bacterium was found to be 93.5% homologous to the *Methylosinus trichosporium* OB3b 5S RNA sequence. A type II methanotrophic bacterium, isolated in pure culture from the bioreactor, synthesized soluble methane monooxygenase during growth in a copper-limited medium and was also capable of rapid trichloroethylene oxidation. The bacterium contained the gene that encodes the soluble methane monooxygenase B component on an *AseI* restriction fragment identical in size to a restriction fragment present in *AseI* digests of DNA from bacteria in the mixed culture. The sequence of the 16S rRNA from the pure culture was found to be 92 and 94% homologous to the 16S rRNAs of *M. trichosporium* OB3b and *M. sporium*, respectively. Both the pure and mixed cultures oxidized naphthalene to naphthol, indicating the presence of soluble methane monooxygenase. The mixed culture also synthesized soluble methane monooxygenase, as evidenced by the presence of proteins that cross-reacted with antibodies prepared against purified soluble methane monooxygenase components from *M. trichosporium* OB3b on Western blots (immunoblots). It was concluded that a type II methanotrophic bacterium phylogenetically related to *Methylosinus* species synthesizes soluble methane monooxygenase and is responsible for trichloroethylene oxidation in the bioreactor.

Chlorinated aliphatic compounds are common groundwater contaminants widely used as industrial degreasers, dry cleaning solvents, propellants, and insecticides (4, 40). Some of these compounds, including trichloroethylene (TCE), are suspected carcinogens (20, 23, 33) or are converted in anaerobic aquifers to vinyl chloride, a known carcinogen in animals (41). Microbial transformations of these organic contaminants in groundwater into harmless end products is a favorable alternative to air stripping or sorption onto activated carbon (22). The latter two processes transfer the contaminants to other environments or concentrate pollutants on activated carbon, which must be disposed of safely.

Halogenated organic compounds, including dichloroethanes, dichloroethylenes, 1,1,1-trichloroethane, vinyl chloride, TCE, and chloroform, are transformed into carbon dioxide and chloride by bacteria that contain nonspecific oxygenases (1, 3, 9, 10, 16, 19, 24, 25, 34, 35, 37, 39, 42, 47).

Some methanotrophs, including *Methylosinus trichosporium* OB3b, are capable of synthesizing soluble methane monooxygenase (sMMO) and can oxidize TCE more rapidly than do oxygenases from other microbes (5, 19, 25, 37). This enzyme can be sensitively and specifically detected in whole cells of some methanotrophs because it catalyzes the oxidation of naphthalene to naphthols (5). Methane-oxidizing bacteria are classified into three types: I, II, and X, on the basis of intracytoplasmic membrane fine structure, phospholipid fatty acid content, DNA base composition, carbon assimilation pathways utilized, and other characteristics (12,

45). Only those methanotrophs classified as types II and X have been shown to synthesize sMMO or contain the gene that encodes the B subunit of sMMO (5, 31). Type I, II, and X methanotrophs synthesize a membrane-bound (particulate) MMO. Type II and X methanotrophs synthesize the sMMO during copper-limited growth (25, 29, 32, 37).

Alvarez-Cohen and McCarthy (2) described a mixed culture of bacteria enriched with methane and oxygen from aquifer material from Moffett Field Naval Air Station, Mountain View, Calif. When grown in a bioreactor under methane and nitrogen limitation, this mixed culture rapidly oxidized TCE (0.6 to 1.1 mg · mg of cells⁻¹ · day⁻¹) and chloroform (0.3 to 0.4 mg · mg of cells⁻¹ · day⁻¹). The effects of competitive inhibition of biotransformations by different substrates, the finite transformation capacity of cells, electron donor supply, and product toxicity on transformation rates were used to develop a cometabolic transformation model (2). This culture has been stable for several years.

We set out to test the hypothesis that type II methanotrophs related to *Methylosinus* species would be enriched, produce sMMO, and rapidly oxidize TCE under the conditions used to select the bioreactor culture. It has been difficult to demonstrate that cultures of methanotrophs isolated from environmental samples and mixed cultures were identical to the dominant bacterium in the mixed cultures, because enrichment methods often favor the growth of minor species in a population and the colony-forming efficiencies of most methanotrophs are very low (12, 13). In addition, the number of biochemical characteristics that can be used for phenotypic comparisons of isolates is small in obligate methanotrophs (13). Therefore, we elected to use

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molecular techniques that permitted convenient and precise comparisons of bacteria in pure and mixed cultures.

MATERIALS AND METHODS

Operation of the bioreactor. The initial development of the mixed culture employed in this study has been described by Lanzarone and McCarty (18). Subsequently, the 7.5-liter culture was grown in a 10-liter baffled semicontinuous-gas-feed chemostat operated with a 9-day detention time with one daily cell wasting and liquid medium replacement (1). The growth medium was the mineral salts medium described by Fogel et al. (9). A mixture of 10% methane in air was injected continuously into the reactor bottom at a rate of 280 ml/min. The stirring rate was maintained at 200 rpm. Methane and oxygen concentrations in the liquid phase were monitored intermittently and maintained at 0.02 mg of methane per liter and 3.5 mg of oxygen per liter. The average cell density was 2,500 mg/liter (range, 1,800 to 3,000 mg/liter).

Bacterial cultures and isolation of pure cultures of a methanotrophic bacterium from the bioreactor. *M. trichosporium* OB3b and *M. sporium* were obtained from R. Whittenbury, Warwick University, Coventry, United Kingdom, and Y. A. Trotsenko, Puschino, Russia, respectively.

Cell suspensions from the bioreactor were diluted in 100 ml of a mineral salts medium (7) in 500-ml triple-baffle Erlenmeyer flasks. The culture was incubated at 30°C with an atmosphere of 20% methane–80% air and agitated on a platform shaker (200 rpm, 2.5-cm stroke length). The atmosphere was replenished twice daily. The culture was diluted 1:1,000 into fresh medium after 2 days and allowed to grow overnight. Cell suspensions were spread plated undiluted onto the surface of minimal salts agar medium (7), and the plates were incubated at 30°C with an atmosphere of 20% methane–80% air (27). When single colonies were visible (approximately 0.5 mm in diameter, 3 to 5 days of incubation), they were restreaked onto the same medium and incubated again. These procedures were repeated several times until a single colony type was present on the plates. The colonies were grayish-white and opaque and were shown to require methane for growth. The colonies oxidized naphthalene to 1- and 2-naphthol, indicating that they were capable of synthesizing sMMO (5). This bacterium, designated *Methylosinus* strain LAC, has been deposited with the National Collection of Industrial Bacteria, Aberdeen, United Kingdom.

Phase-contrast microscopy. Slides were coated on one side with molten 1.5% agar dissolved in 0.15 M NaCl. A cell suspension (10 μ l) was added to the surface of the slide after the agar had hardened. A coverslip was used to cover the cells. After cells had adhered to the agar (approximately 30 min), they were examined by phase-contrast microscopy and photographed with a Zeiss microscope equipped with a Zeiss MC 63 photomicrographic camera attachment (Carl Zeiss Instruments, Inc., Batavia, Ill.).

Isolation of nucleic acids, blotting, and hybridization. Total RNA was isolated from bioreactor samples and frozen cells of other bacteria by the method described by Tsien et al. (36). The RNAs, diluted in 0.01 N NaOH– 10^{-3} M EDTA, were transferred onto nylon membranes (Zeta-probe; Bio-Rad Laboratories, Richmond, Calif.) by using a Bio-Rad slot-blotting device. The blots were rinsed briefly in 2 \times SSC (1 \times SSC is 0.15 M NaCl plus 0.015 M sodium citrate, pH 7.0)–1% sodium dodecyl sulfate (SDS) and air dried. The RNAs were bonded to the membranes by being baked in a

vacuum oven for 30 min at 80°C. The membranes were stored at room temperature in sealed plastic bags until used.

The membranes were sealed in plastic bags with 100 μ l of prehybridization solution (0.9 M NaCl, 50 mM sodium phosphate [pH 7.0], 50 mM EDTA [pH 7.2], 0.5% SDS, 10 \times Denhardt's solution [21], and 100 μ g of sheared and denatured herring DNA per ml) per cm². The membranes were incubated for 2 h at 45°C. Radioactive ³²P-labelled oligonucleotide probes prepared as described by Tsien et al. (36) were added to the bags, and incubation was continued for 16 h at 42°C for probe 9 α , 42°C for probe 10 γ , and 37°C for the eubacterial probe.

The hybridization solution and filters were removed from the plastic bags, and the membranes were washed once in 3 \times SSC–10 \times Denhardt's solution–5% SDS–25 mM sodium phosphate (pH 7.5) for 30 min at 42°C and then twice for 30 min each time in 1 \times SSC–1% SDS at 49°C for probes 9 α and 10 γ and at 42°C for the eubacterial probe.

Fixation and in situ hybridization of whole cells with fluorescent oligonucleotide probes. Preparation of microscope slides, fixation of cells, hybridization reactions, and examination of cells by epifluorescence microscopy were performed precisely as described by Tsien et al. (36).

Isolation and sequencing of 5S rRNA. Total RNA was isolated by the acid-phenol method described above. Approximately 15 μ g of RNA was incubated with [5'-³²P]CDP by using bacteriophage T4 RNA ligase (8). Labelled 5S rRNAs were fractionated by high-voltage electrophoresis through 8% polyacrylamide gels (8). Subsequently, the 5S RNAs were eluted and the one present in the largest quantity was sequenced by the chemical method described by Peattie (28), with minor modifications (8). Sequencing gels (60 cm by 0.19 mm) containing 8, 12, or 20% polyacrylamide were run at 4,500 V until the running dye reached the end of the gel. Sequencing of 16S rRNAs was performed precisely as described by Tsuji et al. (38).

Phylogenetic analysis. 5S rRNA sequences were aligned as described by Wolters and Erdman (48), and tentative phylogenetic trees were constructed by using the novel maximum-topological-similarity method (6, 49).

Pulsed-field gel electrophoresis. A mid-exponential-phase culture of cells grown in mineral salts medium with methane as the sole carbon source (40 to 100 ml containing 10¹⁰ cells) was centrifuged at 6,000 \times g for 20 min at 4°C. The cells were washed once with Pett-IV buffer (10 mM Tris-HCl, 1 M NaCl, pH 7.6) and resuspended in 1 ml of the same buffer. The cell suspension was prewarmed to 40°C before being mixed with a 2% (wt/vol) solution of InCert agarose (FMC Bioproducts, Rockland, Maine) at 40°C. The cell suspension, 200 μ l per slot, was distributed into a mold supplied with the CHEF-DR-II system. The mold was cooled on ice for 15 to 20 min, and the hardened agar inserts were added to 200 μ l of EC lysis solution (30).

The hardened agar inserts were treated as described by Smith et al. (30) to release DNA from the agar-embedded bacterial cells. The inserts were treated for 2 h with 1 ml of phenylmethylsulfonyl fluoride in TE buffer (10 mM Tris-HCl, 0.1 M EDTA, pH 7.4) and washed three times with 1 ml of TE for 12 to 16 h with gentle shaking at room temperature.

Five units of *Ase*I restriction endonuclease per μ g of DNA in 500 μ l of the restriction buffer supplied by the manufacturer was added to the insert in a microcentrifuge tube and incubated for 16 h at 37°C. The insert was washed once with ES buffer and once with ESP buffer (30) for 2 h each time with gentle shaking at 50°C.

A solution of 1% (wt/vol) SeaKem LE agarose (FMC

Bioproducts) in $0.5\times$ TBE (0.05 M Tris, 0.05 M boric acid, 0.1 mM disodium EDTA, pH 8.0) was used to prepare a gel (12.5 by 14 cm). One-fourth to one-eighth of a DNA insert was added to a well in the agarose gel. Lambda concatemer DNA, *Saccharomyces cerevisiae* chromosomal DNA (both from Bio-Rad Laboratories), and lambda bacteriophage DNA digested with restriction endonuclease *Hind*III were used as size markers. Electrophoresis was performed by using a CHEF-DR-II pulsed-field electrophoresis system (Bio-Rad Laboratories). The buffer employed was $0.5\times$ TBE at 14°C for 30 to 40 h. The pulse time varied continuously from 0.1 to 0.2 s. A constant voltage (200 V) was used. After electrophoresis, the gel was stained for 1 h with a $0.5\text{-}\mu\text{g}\cdot\text{ml}^{-1}$ solution of ethidium bromide and photographed.

Southern blotting and hybridization. The agarose gel was soaked in 200 ml of 0.25 N HCl for 20 min and washed twice with 200 ml of 0.5 M NaOH–1.5 M NaCl (20 min each time). Transfer of the DNA to Nytran nylon membranes (Schleicher & Schuell, Inc.) was performed as described by Maniatis et al. (21). After transfer, the membrane was soaked in 200 ml of $5\times$ SSC for 20 min, rinsed once in 200 ml of $2\times$ SSC for 10 min, and air dried.

A cloned DNA fragment containing the sMMO-B component gene from *M. trichosporium* OB3b was labelled with ^{32}P by nick translation using a kit and procedures provided by Bethesda Research Laboratories (Gaithersburg, Md.).

Hybridization reactions and radioautography were performed as described previously (36).

SDS-PAGE and Western blot (immunoblot) analysis for detection of hydroxylase components of sMMO. Cell proteins in extracts of methanotrophs were separated by discontinuous SDS-polyacrylamide gel electrophoresis (PAGE) (17). After Western blotting, hydroxylase component subunits that cross-reacted with antibodies prepared against purified sMMO hydroxylase from *M. trichosporium* OB3b were detected as described by Tsien et al. (37).

RESULTS

Characteristics of the mixed culture present in the bioreactor. The mixed culture in the bioreactor was salmon pink and grew attached to the reactor walls, as well as suspended in the medium. Phase-contrast microscopy revealed a nonmotile, crescent-shaped bacterium with phase-dark granules, fewer numbers of a prosthecate bacterium resembling *Hyphomicrobium* species (14), and highly motile rod-shaped bacteria (Fig. 1A). The large, crescent-shaped bacterium often formed rosettes (Fig. 1B). The methanotrophic bacterium isolated in pure culture from the bioreactor was morphologically indistinguishable from the crescent-shaped bacterium that was present in the bioreactor.

In situ hybridization with fluorescent signature probes. Synthetic fluorescent oligonucleotides labelled with fluorescein isothiocyanate (36) were used in *in situ* hybridization with formaldehyde. The results of these experiments (Fig. 2) showed that the larger crescent-shaped bacterium hybridized with fluorescently labelled probe 9α , which specifically hybridizes to type II methanotrophs (36). Probe 10γ , which is homologous to 16S rRNA signature sequences of type I and X methanotrophs, did not hybridize with any cells in the bioreactor culture. Cells from a pure culture known as *Methylosinus* strain LAC also hybridized with the 9α signature probe (data not shown).

Production of sMMO by cells growing in the bioreactor. Antibodies prepared against sMMO of *M. trichosporium* OB3b have been employed to detect sMMO components in

several type II methanotrophs and *Methylococcus capsulatus* Bath after denaturing SDS-PAGE and Western blotting (37a). The data in Fig. 3 show that Western blots of crude extracts of cells from the bioreactor contain proteins that cross-react with anti-sMMO antibodies and have molecular weights similar to those of the α and β subunits of the hydroxylase component of the sMMO of *M. trichosporium* OB3b. The purified hydroxylase component has subunits with molecular masses of 54, 43, and 23 kDa for the α , β , and γ subunits, respectively (11). The γ subunit was only weakly visible in the Western blots in crude extracts prepared from *M. trichosporium* OB3b cells and extracts prepared from cells obtained from the bioreactor shown at the right of Fig. 3, but the γ subunits were visible in the PAGE gel stained with Coomassie blue (Fig. 3, arrow).

The hydroxylase subunits of the sMMOs of nearly all of the methanotrophs that produce this enzyme have approximately the same molecular weights (11, 26, 29, 31, 32). Antibodies prepared against sMMO hydroxylase used to detect sMMO hydroxylase subunits on Western blots were suitable for detection of these proteins in crude extracts, as well as in purified enzyme preparations (Fig. 3). The molecular weights of the hydroxylase α and β subunits in extracts from the *M. trichosporium* OB3b and the bioreactor cultures appeared to differ slightly (Fig. 3). The mass of the hydroxylase β subunit from the bioreactor culture was slightly less than that of the β subunit from *M. trichosporium* OB3b. The pure culture of *Methylosinus* strain LAC produced sMMO hydroxylase subunits that were identical to those of the bioreactor culture on Western blots (data not shown).

Detection of the sMMO-B gene in DNA samples. DNA samples prepared from *M. trichosporium* OB3b cells, cells harvested from the bioreactor, and cells of *Methylosinus* strain LAC were incubated with the infrequently cutting restriction enzyme *Ase*I. The restriction fragments produced were then separated on agarose gels by pulsed-field gel electrophoresis. The restriction fragments produced by *Ase*I digestion of total DNAs from the bioreactor culture and the pure culture of *Methylosinus* strain LAC were similar or identical in size (Fig. 4). After Southern blotting, the membrane-bound DNAs were hybridized with the ^{32}P -labelled cloned DNA fragment that encodes the *M. trichosporium* sMMO-B component gene. The *Ase*I restriction fragment of *M. trichosporium* OB3b that hybridized to the sMMO-B gene probe was approximately 180 kb long, while the fragments from DNAs prepared from bioreactor cells and *Methylosinus* strain LAC cells were approximately 85 kb long (Fig. 4). The *Ase*I restriction fragments of all different type II methanotrophs that hybridize to the sMMO-B gene probe were readily distinguishable by size (36). The restriction fragments from DNAs of bacteria that are present as a small fraction of the total bioreactor population are not observable in lane 2 of Fig. 4.

Phylogenetic relationship of bioreactor culture. The sequences of the major 5S rRNA isolated from the bioreactor culture and two other type II methanotrophs are shown in Fig. 5. The major 5S rRNAs of the mixed bioreactor culture and *M. trichosporium* OB3b are 95.3% similar in sequence. The phylogenetic relationships between the bacterium that is the most abundant in the bioreactor and some type I, II, and X methanotrophs are illustrated in Fig. 6.

The sequence of 16S rRNA from *Methylosinus* strain LAC was 92 and 94% homologous to the 16S rRNA sequences from *M. trichosporium* OB3b and *M. sporium*, respectively.

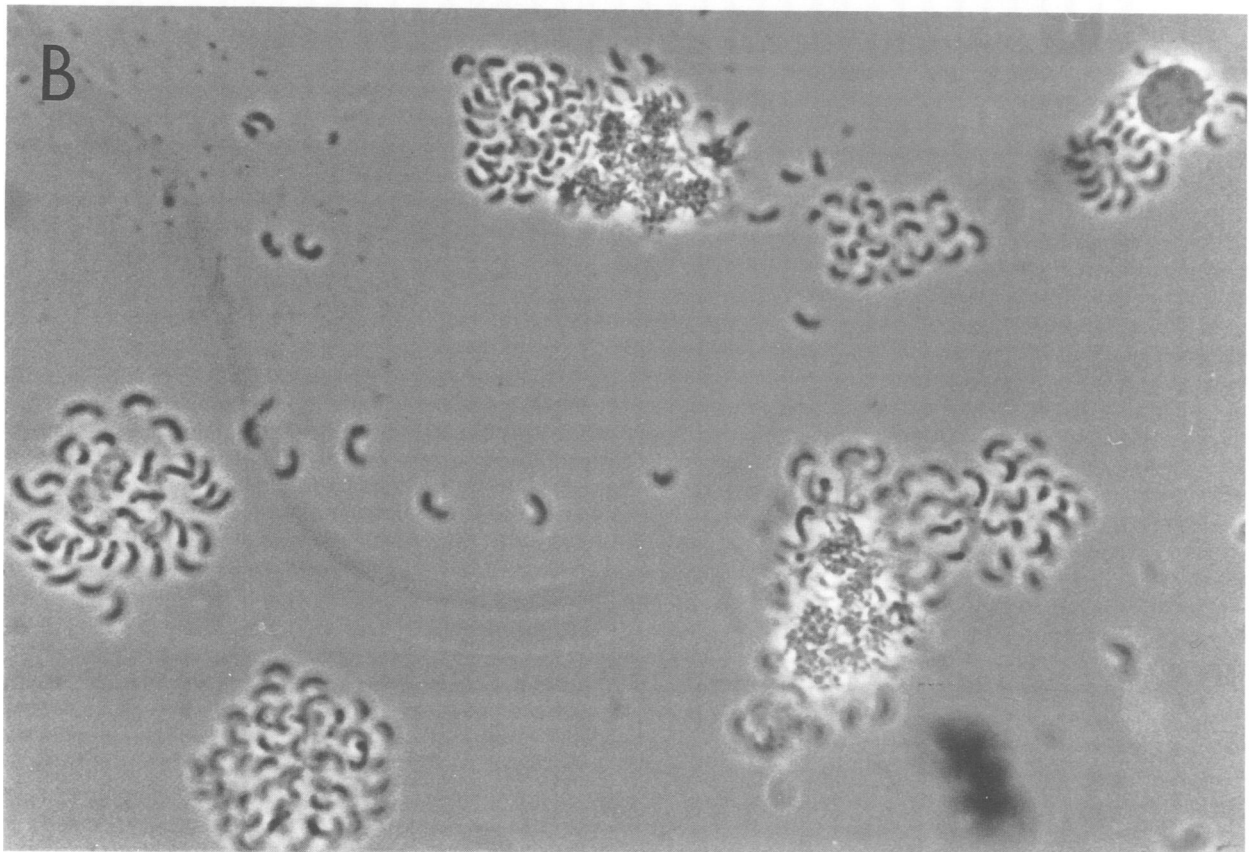
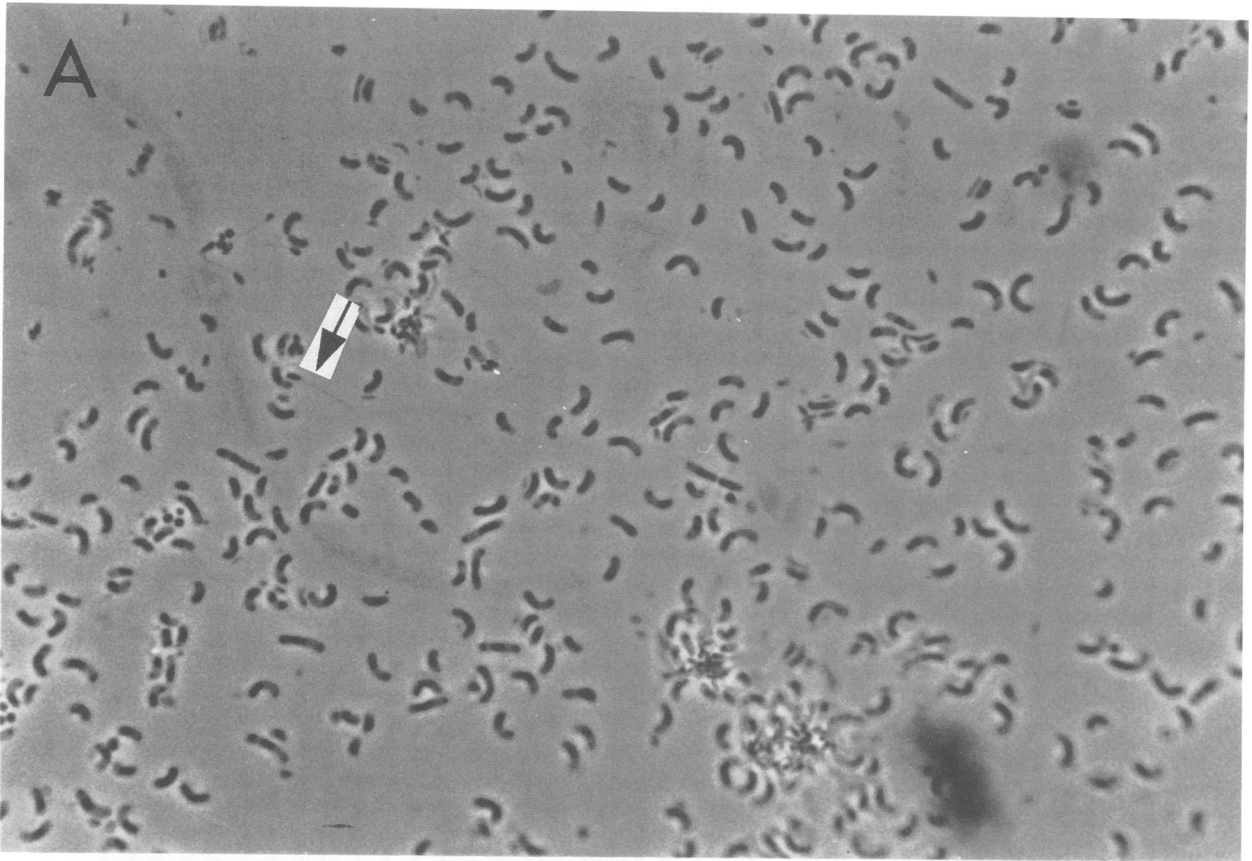


FIG. 1. Phase-contrast photomicrographs of cells of the mixed culture in the bioreactor. (A) Stalked cells morphologically similar to *Hyphomicrobium* species were present in small numbers (arrow). (B) Clumps of crescent-shaped cells similar to *Methylosinus* species (45) were present when cells were placed on agar. Cells of *Hyphomicrobium* species were clustered within groups of larger cells.

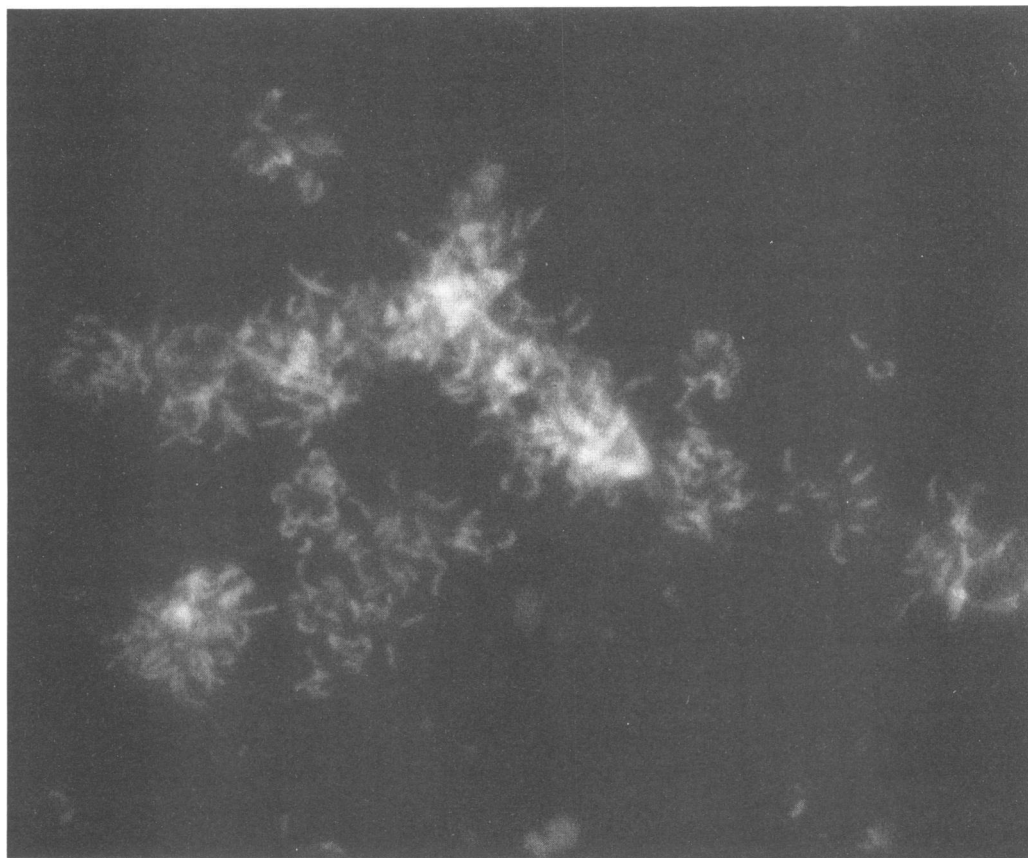
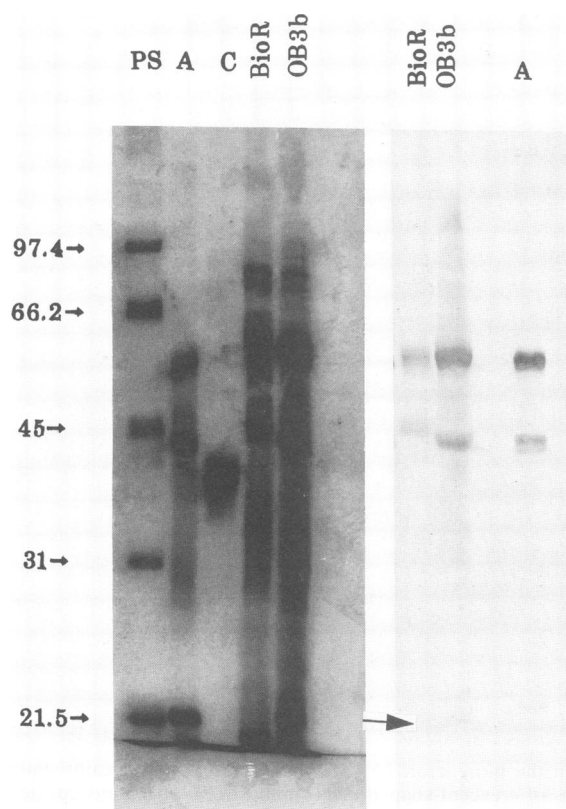


FIG. 2. Fluorescence photomicrograph of fixed cells of the bioreactor mixed culture hybridized with fluorescein-labelled signature probe 9 α .



DISCUSSION

A mixed culture of bacteria that was grown with methane as the sole source of carbon and energy and was capable of rapid transformations of TCE and chloroform contained a type II methanotroph similar to bacteria of the genus *Methylosinus* as the dominant bacterium. During growth in a bioreactor, this bacterium produced sMMO. These conclusions are supported by the following observations. The major component of the mixed culture was a large, crescent-shaped bacterium similar in morphology to *M. sporium* (43, 45). This bacterium was morphologically indistinguishable from *Methylosinus* strain LAC, which was isolated in pure culture from the bioreactor. Bacteria in the bioreactor produced protein components of the sMMO detected by Western blotting and contained sequences complementary to the cloned sMMO-B component gene from *M. trichosporium*.

FIG. 3. Western blot analysis for detection of subunits of the hydroxylase components of sMMO. (Left) polyacrylamide gel of crude extracts stained with Coomassie blue. (Right) Western blot treated with anti-MMO hydroxylase antibodies. The antibody-antigen conjugates were detected as described by Tsien et al. (37). Lanes: PS, molecular size standards (kilodaltons); A, purified hydroxylase component of sMMO from *M. trichosporium* OB3b; C, purified reductase component of sMMO from *M. trichosporium* OB3b; OB3b, crude extract of *M. trichosporium* OB3b; BioR, crude extract of cells from the mixed culture in the bioreactor. The arrow indicates the position of the hydroxylase γ subunits on the Western blots.

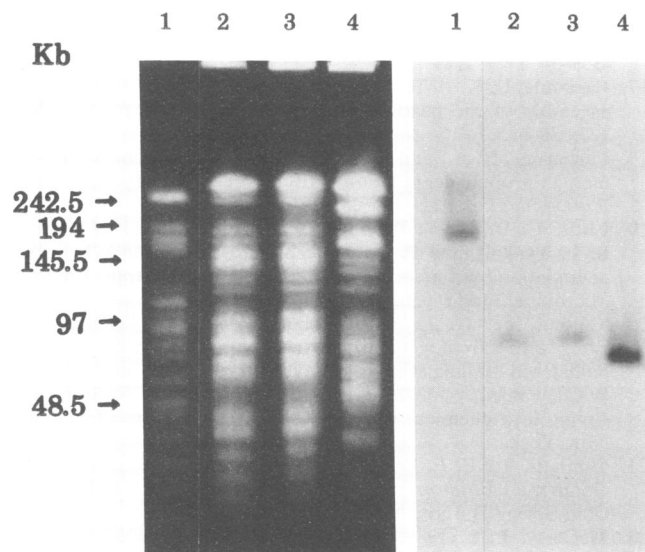


FIG. 4. Detection of restriction fragments complementary to the cloned MMO-B gene of *M. trichosporium* OB3b on Southern blots of agarose gels. The fragments were separated by pulsed-field electrophoresis. Lanes: 1, *M. trichosporium* OB3b DNA; 2, DNA from the bioreactor mixed culture; 3, DNA from *Methylosinus* strain LAC; 4, DNA from a methanotrophic bacterium isolated from *Lemna minor* plants. (Left) Agarose gel stained with ethidium bromide showing restriction fragments produced by treatment with restriction endonuclease *AseI*. Numbers on the left indicate fragment sizes in kilobase pairs. (Right) Southern blots of the agarose gel were hybridized with the [³²P]sMMO-B component gene probe and exposed to X-ray film.

OB3b. The pure culture of *Methylosinus* strain LAC isolated from the bioreactor also contained DNA sequences homologous to the sMMO-B component gene. The hybridizing *AseI* restriction fragment from *Methylosinus* strain LAC was indistinguishable in size from the hybridizing fragment isolated from cells in the bioreactor. The sizes of the *AseI* restriction fragments produced by digestion of DNA isolated from *Methylosinus* strain LAC and the cells in the bioreactor were clearly distinguishable from those of other methanotrophs we have examined.

The most abundant bacterium in the bioreactor, *Methylosinus* strain LAC, was a type II methanotroph phylogenetically related to *Methylosinus* species. It was clearly distinguishable from other *Methylosinus* species by the sizes of *AseI* restriction fragments that were complementary to the *M. trichosporium* OB3b sMMO-B gene. Protein components of the sMMO and DNA sequences complementary to the

1.	<i>Methylosinus sporium</i> st. 5W:	NGCCUGGUGG	CUUUAGCGAA	GCGAUCAGAC
2.	<i>Methylosinus trichosporium</i> OB3b	GGCAUGGUGG	CUUUAGCGAA	GUGAUUAGAC
3.	5S rRNA isolated from the bioreactor culture	AGCCUGGUGA	CUUUAGCGAA	GUGAUOAGAC

1.	CCGAUCCCAU	CCCGAACUCG	GCCGCUAAAC	GCUCCAGCGC	CAAUGGUACU	AUGUCUCAAG
2.	CGUAUCCCAU	CCUGAACUCG	GCCGCUAAAC	GCUCCAGCGC	CAAUGGUACU	AUGUCUCAAG
3.	CGUAUCCCAU	CCCGAACUCG	GCCGCUAAAC	GCUCCAGCGC	CAAUGGUACU	AUGUCUCAAG

1.	ACCUGGGAGA	GUAGGUGGUC	GCCAGGCUN			
2.	ACCUGGGAGA	GUAGGUGGUC	GCCAGGCNN			
3.	ACCUGGGAGA	GUAGGUGGUC	GCCAGGCUN			

FIG. 5. Sequences of 5S rRNAs isolated from two type II methanotrophs and bacteria in the bioreactor mixed culture.

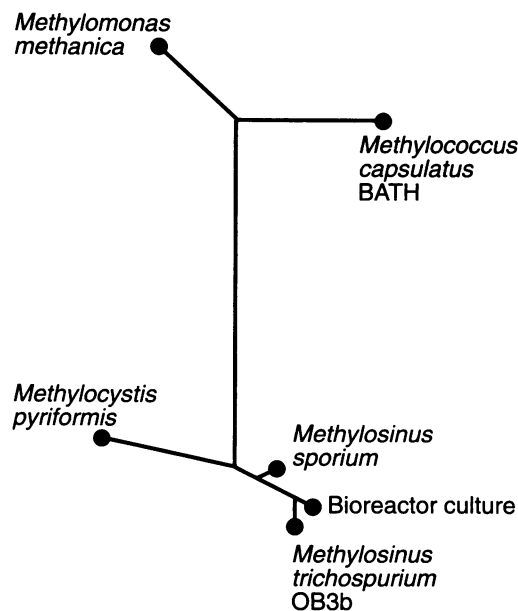


FIG. 6. Phylogenetic relationships of selected methanotrophic bacteria based on 5S rRNA sequences. *Methylosinus* and *Methylococcus* species are obligate type II methanotrophs (13, 44). *Methylosinus trichosporium* OB3b is an obligate type I methanotroph, and *M. capsulatus* Bath is a type X methanotroph (46). The phylogenetic tree is based on comparative sequence analysis of the bacteria as described by Yushmanov and Chumakov (49).

sMMO-B component gene have been detected only in some type II methane-utilizing bacteria (13, 37a). DNA extracted from *M. capsulatus* BATH, a type X methanotrophic bacterium (46), does not contain sequences complementary to the *M. trichosporium* OB3b sMMO-B gene.

sMMO components are synthesized in *M. trichosporium* OB3b cultures during copper-limited growth (25, 32, 37). Cells in the bioreactor also synthesized these proteins. The medium supplied to the bioreactor contained 10 μ g of copper chloride per liter (0.75 μ M) added to deionized water (1). The biomass reached an average cell density of 2,500 mg/liter (33 g cells per μ mol of copper). The sMMO of *M. trichosporium* OB3b and *M. sporium* was synthesized when the biomass-to-copper ratio exceeded 4.0 g/ μ mol (37). Therefore, it was anticipated that sMMO would be synthesized if type II methanotrophs related to *Methylosinus* species were present in the bioreactor.

Methylosinus strain LAC synthesized sMMO, as evidenced by the ability of cells to convert naphthalene to 1- and 2-naphthol. This reaction is catalyzed only by bacteria containing sMMO (5).

We have detected rates of TCE oxidation above 100 μ mol/h/g of cells by methane-oxidizing cultures only when type II methanotrophs were present and only under conditions that permitted expression of sMMO (5, 37). Type I methanotrophs containing the particulate MMO have been reported to oxidize TCE at slower rates (15, 19). The high rates of TCE oxidation in the bioreactor described by Alvarez-Cohen and McCarty (1) have also been observed with the pure isolate *Methylosinus* strain LAC when it is grown under conditions that permitted synthesis of sMMO (1). This further indicates that *Methylosinus* strain LAC is the predominant bacterium responsible for TCE oxidation in the bioreactor. This bacterium represents a new isolate

different from previous isolates classified as *Methylosinus* species.

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